

Important Notice—Barcoded Mirror Slides

The enclosed microarrays from the Vanderbilt Microarray Shared Resource have been prepared with a proprietary printing buffer printed on a mirrored substrate. The background level and sensitivity of these arrays are outstanding when the proper prehybridization, hybridization and washing protocols are followed. Total dye amounts as low as 6pmol have given excellent results while dye amounts in excess of 20pmol may cause high background. Dye amounts between 12 and 20pmol have given excellent results in laboratory testing. As a point of reference, a typical direct incorporation of Cy dye on 30ug of total RNA will generally yield 50-100pmol of incorporated dye. Yields from indirect protocols are generally higher. **Important note:** Each print run of our microarrays has a unique layout. Any slides with 48 grids (Mouse 22K and Human Oligo arrays) have the barcode label placed on the back of the slide. Printed spots are visible and should be checked to ensure proper slide orientation before use. To determine the version of chip you are using, please see the paperwork that was sent with the arrays or visit our website (www.vmsr.net). Descriptions of all arrays, protocols and genelists are available along with a tool to decode the barcode on each slide.

Full protocols for labeling, hybridization and washing and array layout files are available at
<http://www.vmsr.net/support/downloads.htm>

Prehybridization:

Slides are provided in a semi-processed state with the DNA fixed to the glass but the salt spots still clearly visible. Arrays must be prehybridized prior to use to wash away any shipping dust and unbound material.

1. Prepare a solution of 0.2% SDS at RT. Arrange the slides in a slide rack and vigorously plunge the slides into the SDS solution. This vigorous washing rapidly removes any unbound material and prevents “comet tails” from forming on the slides
2. Immediately transfer the slides to warm prehybridization solution (5xSSC; 1% Bovine Serum Albumin (BSA); 1% SDS) and prehybridize for 45 minutes at 55°C.
3. Using 5-7 changes of water, vigorously wash the prehybridized slides. It is absolutely imperative ALL prehyb material is removed from the slides. You cannot wash too much. Following the final wash, rinse the slides in isopropanol and air dry. Once the slides are dry, there should be no visible material on the slides. Any visible material may lead to high background levels.

Hybridization:

1. Prepare a **2x** hybridization solution containing 50% deionized Formamide, 10X SSC, 0.2% SDS.
2. Depending on the array and size of coverslip, resuspend your target material as follows, apply to array and hybridize for 16 hours at 42°C:

Coverslip Size (cat #)	Volume Water	Volume 2x Hyb Sol	Vol 10mg/ml PolyA RNA	Total Hyb Vol
24x40mm (2-4959)	40ul	40ul	1ul	81ul
24x60mm (2-4733)	50ul	50ul	1ul	101ul

Hybridization Volumes above are estimates. Adjustments may be required for optimal performance. Lifterslips from Erie Scientific are recommended for all hybridizations. Catalog numbers are given above.

Washing:

Following hybridization, arrays should be washed with three successive washes of 2xSSC-0.1% SDS, 1xSSC and 0.1xSSC. First wash is done at 55°C, second and third at RT.